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Contract NASW-1407

GNOTOBIOTIC SURVEY REPORT

JULY 1966

MARTIN COMPANY
DENVER, COLORADO

VOY-CR-66-5

Contract NASW-1407

GNOTOBIOTIC SURVEY REPORT

July 1966

Approved

A. A. Rothstein
Program Director
Sterile Insertion Program

MARTIN COMPANY
A DIVISION OF MARTIN MARIETTA CORPORATION
Denver, Colorado

FOREWORD

This report is issued in compliance with the requirements set forth in Contract NASW-1407.

CONTENTS

SUMMARY

This report on gnotobiotic technology has been prepared in three chapters. Chapter I contains a concise summary of the historical development and present status of this field. Phillip Trexler prepared this chapter based on his comprehensive knowledge of the multifaceted activities in this area.

Selected abstracts related to gnotobiotic technology are provided in Chapter II. These abstracts, selected from the Martin files on sterilization technology, have been included to show the scope of gnotobiotic work as well as to identify valuable papers.

Résumés and addresses of personnel preparing the abstracts in this report are contained in Chapter III. Recipients of this report who are interested in specific abstracts are invited to contact the abstractor to discuss the technical content.

I. DEVELOPMENT OF GNOTOBIOTIC TECHNIQUES

Sterile insertion techniques in conjunction with the production and use of the higher animals in the absence of microbic contamination have requirements that are similar to those involved with the production and maintenance of sterile spacecraft. A high order of microbiological security is required when gnotobiotic colonies are maintained continuously and a great variety of supplies introduced into the sterile area. Food, bedding, water, and a variety of instruments such as balances are required for the use of gnotobiotic animals. The application of these techniques with the larger animals such as sheep and swine requires the development of mechanical techniques for transferring large objects from one isolator to another. While the mechanical problems involved are not as complex as those anticipated with spacecraft, they are nevertheless considerably more involved than the procedures encountered in the microbiological laboratory. For this reason, an examination of the sterile insertion techniques as developed for gnotobiotics may be instructive in estimating the utility of the method.

The first germfree animals, guinea pigs, were reported in 1895 by Nuttall and Thierfelder (Ref 1). They succeeded in rearing some animals for two weeks. However the high contaminaton rate was probably caused by the use of ordinary aseptic surgical techniques to caesarean-derive the animals and insert them in the sterile isolator chamber. Once the animals were introduced into the sterile chamber, no further insertions were made. The chamber, as sterilized in the autoclave, contained all the feed that the animals would have.

The sterile insertion techniques as used in the microbiological laboratory and in surgery are essentially an open system in that the workers, obviously carrying microbiological contaminants, are in the sterile area. Contaminations are reduced by control of air currents and prevention of direct contact through the use of sterile gloves, masks, gowns, etc. A closed system for sterile insertion, in which the operator is not in contact with the working area, was first described by Küster in 1914 (Ref 2). However, this method was used for introducing supplies only. The animals (goats) were introduced through an open system.

The first closed insertion technique for introduction of the animal was described by Reyniers and Trexler in 1943 (Ref 3). The animal was introduced through a passageway in the bottom of the isolator (a cylindrical steel tank with gloves, sight ports, air filters). The entire chamber was sterilized with steam under pressure. The lower passageway was sealed off with a cellophane film by manipulations through attached rubber gloves. After the film was in place, the pressure door was opened from the outside. The gravid animal was prepared for surgery, and the disinfected skin of the abdomen was placed against the cellophane film. A hot wire cautery was manipulated within the sterile chamber and an incision made through the cellophane and the skin of the animal. The hot wire sterilized the edges of this opening. The animal was then introduced using standard surgical procedure. The animals were wiped with a germicide and then passed into a second isolator attached to the operating chamber. In this way, the young were removed from the animal and introduced into a rearing chamber without exposure to the open atmosphere or the attendants. Supplies were introduced through an attached passthrough steam sterilizer that serves as a sterile lock. This is the first completely closed isolator system.

A somewhat similar technique was described by Gustafsson in 1948 (Ref 4). With the Gustafsson technique, an incision is made through the surgically prepared skin of the abdomen of the gravid animal. The edges of the skin are then attached to a circular opening in the bottom of the operating chamber. A space between the plastic membrane that seals the operating tank from the surgical area and the abdomen of the animal is decontaminated by a liquid germicide. Surgery is then performed through the liquid seal.

The Reyniers germfree apparatus (Ref 5) and the Gustafsson apparatus (Ref 6) are similar in that the chamber is made of stainless steel with attached rubber gloves for manipulation and a glass sight port. Both were sterilized by steam under pressure. However, they differ in the method used for sterile insertion. Reyniers' apparatus contains small passthrough sterile locks in which steam under pressure is used as a sterilant. With the Gustafsson apparatus, sterile insertion is accomplished by a germicidal bath that traverses the wall of the chamber. The germicide itself is not sporicidal, so a successful operation depends on aseptic procedures. Insertion is accomplished by placing the sterilized object within a bottle that was then passed through the liquid trap. Though the sterile insertion procedure was thought to be the most vulnerable step in the operation, no contaminations were traced to failures in this step. A total of 1260 insertions were reported

with no failures. A survey of contaminaton is reported in Table 1. All of the contaminants reported are preventable. A report on contamination experience in colony operations using the Reyniers apparatus is reported in Table 2. One contamination due to defective apparatus is reported in the total of 1274 sterile insertions.

Neither the Reyniers nor the Gustafsson apparatus appears directly applicable to the fabrication of spacecraft because the heavy metal chambers places a severe mechanical constraint on the design of chambers for housing spacecraft. Flexible film apparatus (Ref 7) appears to be more adaptable to protecting large apparatus without placing undo constraint on manipulations and sterile insertion. A comparison of contamination rates between the flexible film apparatus and Reyniers' system on similar operations indicates that the flexible film apparatus is somewhat more reliable (Ref 8). This indicates that flexible film when used with laboratory animals does not present a puncture hazard. This must be reevaluated for different applications. Manipulations usually require the use of rubber gloves to protect the hands of the operator. Experience in the use and production of animals indicates that the glove is more susceptible to puncture than the chamber wall; puncture hazard is more of a concern with the gloves than with the chamber walls.

Two new sterile insertion techniques (peracetic acid lock and split-seam transfer) were devised to take advantage of the light weight and mobility of the flexible film isolators. A peracetic acid sterile lock consists of a passthrough chamber in which surface sterilization is accomplished by a peracetic acid fog or spray (Ref 9). This has been the method generally used with flexible film isolators and has given no contamination difficulty with peracetic acid as a surface sterilant. Recommended procedures involve wetting of all surfaces with a spray of 2% peracetic acid and wetting agent followed by a holding period of 20 minutes. The original tests were run using 5000 spores of the most resistant species available (Bacillus stearothermophilus). Unreported data indicate that 0.1% peracetic acid will inactivate these spores in the vapor phase within 20 minutes. The recommended concentration, therefore, is 20 times the minimum required. The liquid phase will inactivate these spores in less than two minutes, which is 1/10 the recommended holding period. It is difficult to estimate the resistant spore population in a sterile lock. However, in hundreds of tests in the laboratory, these resistant spore populations have been more resistant than the native microbes in the isolators and chambers tested. This indicates that the recommended procedure for peracetic acid operated sterile lock has a great built-in safety factor. D values have not been determined because of the difficulty of removing spores quantitatively from surfaces. Little work has been done with spore suspensions, since it was felt that our primary objective is surface sterilization.

Table 1 Survey of the 5 Contaminations Out of 41 Runs: September 1956 to March 1958 (over 2304 isolator-days)*

Cause	Contaminants
Glove Puncture by Rat Bite (rats loose in apparatus)	Animals Sacrificed
Glove Puncture by Rat Bite (rats loose in apparatus)	Animals Sacrificed
Leak in Food Autoclave (overlooked at testing)	Mold
Leak in Food Autoclave (overlooked at testing)	Mold
Leak in Glove Sleeve (overlooked defect of manufacture)	Cocci, Rods, and Fungi

*From Ref 6, p 27.

Table 2 Group 1 Operations of Germfree Isolators with C3H Mice, Swiss Mice and Rats (March 1955 to October 1957)*

Germfree Isolators Used	6
Supply Lock Passages	1210
Initial Isolator Sterilizations	32
Isolator to Isolator Passages	64
Contaminations Due to Apparatus Failure†	1

*From Ref 5, p 76.

†This is the only failure in these experiments and was due to a warped outer supply lock door that failed when a vacuum was created in the lock following sterilization.

Split seam transfer was described as a method of introducing supplies into a flexible film isolator (Ref 10). This method resembles that used to introduce the fetus into the isolator (Ref 3). Supplies to be introduced are steam sterilized in a flexible film package. This package is then attached to the wall of a flexible film isolator either by heat sealing or cementing. A passageway is then cut through the sealed area by a hot wire cautery and the supplies introduced. This method has been used to maintain a colony of gnotobiotic mice for six months without contamination (Ref 8). This method has also been used to introduce supplies to an isolator used with human surgery (Ref 11). No contamination has been reported when using this method. However, it has not been used very widely in comparison with the peracetic acid sterilized lock.

Contamination experience with two commercial animal colonies as they started to use isolators has been reported as averaging one per 252 isolator-days (Ref 12), and one per 166 isolator-days (Ref 13). The source of one contamination was not located. None of the others were traced to failure of the sterile insertion procedure. None of the operating crew were trained in sterile technique.

Contaminations in one of the commercial colonies following the initial training period is reported in Tables 3 and 4. No contaminations occurred during the period reported in Table 3. The contaminations that subsequently occurred (Table 4) were a result of economy measures. Apparently contamination rates approach zero if isolators are carefully maintained. A nucleus colony of gnotobiotic mice have been maintained continuously for more than eight years without a single contaminant.

The objectives of practically all of the gnotobiotic operations are to produce and/or use laboratory animals in a variety of studies. For this reason, tradeoffs are made between security and economy of operation. The highest degree of microbiological security (Ref 7) can be made if physical means are used to monitor the integrity of the isolating barrier. Microbiological test procedures are of limited value for demonstrating the sterility of an individual isolator. They are useful in establishing operating procedures and detecting failures, though negative results are not conclusive. The experience gained in the long-term maintenance of isolators with materials, such as axenic animal colonies, that are sensitive to microbiological contamination should provide an estimate of the reliability of the method.

Table 3 Contamination Report, September 20, 1963 to May 26, 1964 (65 isolators in continous operation)*

Contaminations	0
Glove entries approximately	17,000
Sterile Insertions (approximate) (The Charles River Breeding Lab, Inc.)	6,000

*From Ref 14.

Table 4 Contamination Report, September 20, 1963 to January 31, 1966 (65 isolators in operation)*

Source	No. Isolators
Trainee Error	3
Apparatus Development	11
Glove Punctures	7
Isolator Film Punctures	3
Episode A Sterile Drum Damaged	17
Episode B Feed Sterilization Failure	15
Unknown	3
Total	59
Glove Entries (approximate)	60,000
Sterile Insertions (approximate)	20,000

*From Ref 14.

II. SELECTED GNOTOBIOTIC ABSTRACTS

This chapter contains abstracts relating to gnotobiology, sterilization techniques, and microbiology and space.

A. GNOTOBIOLOGY

1. D. Carter and A. Einheber: "A System for Providing Surgical Anesthesia for Germfree Rodent," J. Appl. Physiol. 20(3): 571-572 1962

A system to provide anesthesia for experimental surgical procedures on germfree rodents within a germfree surgical isolator is described. The preselected air-halothane mixture is sterilized by passage through spun-glass filters.

2. Thomas D. Luckey: "Effect of Microbes on Germfree Animals," Advances in App. Microbiology, Vol 7, 1965

Nuttall and Thierfelder performed the first experiment with germfree animals in 1894. Trexler and Reynolds (1957) developed inexpensive isolators initiating a great increase in germfree research. Total count of micro-organisms in human feces is 10"/gm (10% of human feces is living bacteria). Assuming the fecal content of the colon of man weighs 1.5 kg, 150 (0.15 kg) gm of micro-organisms would be present. Each micro-organism digesting to food during a 10 to 60 minute life span. If it digests its body weight in 1 minute - 0.15 kg x 1440 minutes = 216 kg of food would be digested per day. The above figure is 100 times the total content of the colon. Humans eat 1.5 kg/day; therefore, the bacteria have the potential to process 100 times as much food. However, there is considerable restriction on the activity of the micro-organisms in the alimentary canal. An important factor in the microecology of the alimentary tract is continued reinoculation.

3. Mark Chatigny: "Protection Against Infection in the Microbiological Laboratory: Devices and Procedures," Advances in App. Microbiology, 3 (1961)

4. Arthur W. Phillips and James E. Smith: "Germfree Animal Techniques and Their Application," Advances in App. Microbiology, 1 (1959)

5. E. M. Tuff, R. J. Rozntree, and L. H. Frommhagen: "Enteric Infections in Gnotobiotic Pigs," Bact Proc (1966)

The animals tolerated monocontamination with E. Coli or S. Typhimurium rough I strain (makes the polysaccharide side chain responsible for the 1, 4, 5, and 12 Salmonella 0 antigens but does not attach it to the cell wall). Introduction of the semi-rough strain resulted in death in about two to three days (semi-rough strain makes and attaches side-chain components whose polysacchride content is about 1/9 that of the side chains of the fully smooth parent strain). Introduction of the rough II (does not make side chain) resulted in death in about nine days.

6. M. Lev: "A Device for the External Supply of Sterile Water and a Simple Air Sterilizing Filter for Germfree Units," J. Appl. Bact., 27 (1) 41-44

A device for the safe external supply of sterile water and a simple air filter are described. This type of filter is regularly used to supply sterile air to germfree animals. The apparatus is supplied at a rate of 15 to 20 liters of air/minute, and the animals are reared germfree for periods up to seven months.

7. J. R. Thompson: "Current Applications of Biological Science VI. Gnotobiotics: The Science of Germfree Life," Bios, 36(2): 71-76 1965

8. W. O'Toole et al.: "Studies of Postmortem Microbiology Using Sterile Autopsy Technique," Arch. Pathol., 80(5): 540-547 1965

Suggest certain revision in commonly accepted ideas concerning postmortem microbiology.

9. J. Heneghan: "Gnotobiotic Dogs for Surgical Research," J. Surg. Res., 6(1): 24-31 1966

New tool for knowledge of the role of bacteria in surgical problems.

10. M. Krichevsky and L. Zern: "A Simple Indicating Device for Low Water Levels in Sterile Water Tanks for Germfree Isolators," Proceedings of the Gnotobiotic Workshop and Symposium, Ohio State University, Columbus, Ohio, July 22 thru 24, 1963

11. P. H. Miller: "A Technique for Maintaining a Sterile Soil: Plant Root Environment and Its Application to the Study of Ameno Acids in the Rhizosphere," Soil Sci., 100(4):267-273 1965

A plant culture unit for the growth of plants in soil with aseptic roots was described.

12. P. C. Trexler and S. M. Levenson: "Application of Gnotobiotic Technology to Human Medicine," Proceedings of the Gnotobiotic Workshop and Symposium, Ohio State University, Columbus, Ohio, July 22 thru 24, 1963

13. P. C. Trexler: "Gnotobiotic Techniques and Their Application to Spacecraft Fabrication," Life Sciences and Space Research II, North-Holland Publishing Company, Amsterdam, 1964

This report discusses the techniques and procedures that have been used to rear animals totally free of viable micro-organisms. States that these techniques are applicable to the assembly of spacecraft subassemblies in a sterile environment, and that this technique would alleviate some of the problems involved with terminal sterilization.

14. Arthur W. Phillips and James E. Smith: "Germfree Animal Techniques and Their Applications," Advances in App. Microbiology, 1:141-173 1959

Contains 113 references.

This report describes methodology of germfree birds and mammals, their characteristics, and some of their applications. Consideration is also given to various means of rendering these techniques more accessible to all interested investigators.

Areas discussed under methods and equipment include high-pressure-type isolators, low-pressure-type isolators (ethylene oxide, peracetic acid, plastic isolators), and shipping isolators (discusses techniques for transportation of germfree animals up to distances of 2000 mi).

15. M. Pollard et al.: "Spontaneous Leukemia in Germfree AK Mice," Proc Soc Exp. Biol Med, 120(1):72-75 1965

Lymphatic leukemia developed spontaneously in germfree AK mice. The characteristics of disease were the same as those observed in the conventional control AK mice. The data indicate that the leukemia agent was transmitted to progeny by congenital routes.

B. STERILIZATION TECHNOLOGY

1. G. Briggs Phillips: Microbiological Contamination Control, Prepared by the Biological Contamination Control Committee, American Association of Contamination Control, 1965

This paper contains a good review of principles, techniques, and procedures used in contamination control. Included in this report are discussions on:

1) Importance of man in contamination control;

2) Microbiological contamination control barrier systems;

3) Sterilizing and decontaminating agents;

4) Standards, measurements, testing, and criteria of control;

5) Areas of application;

6) Stages in achieving microbiological contamination control.

2. A Bibliography on Vapor Phase Disinfectants, Prepared by the Biological Contamination Control Committee, American Association for Contamination Control, 1965

Contains 128 references on vapor phase disinfectants. Includes references on Beta-propiolactone, ethylene oxide, formaldehyde, methylbromide, peracetic acid, and propylene oxide.

3. T. Holme: "Sterilization of Microbiological Media with B-Propiolactone," Biotechnology and Bioengineering, Vol VII 129-132, 1965

A procedure for sterilization of microbiological media with B-propiolactone has been developed. Special attention was paid to the maintenance of mild conditions to enable the treatment of media sensitive to high temperature or low pH. The maximum temperature allowed was 40°C and automatic neutralization of acid produced during hydrolysis was effected by the use of a titration unit. The apparatus could be used for several successive sterilization cycles without disconnection.

4. M. Rittenbury and M. Hench: "Preliminary Evaluation of an Activated Glutaraldehyde Solution for Cold Sterilization," Ann. Surg. 161(1):127-130 1965

Activated glutaraldehyde solution (Cidex) is very effective, does not require prolonged exposure to effect sterilization, and is practically not irritating or toxic. There is no damage to any equipment exposed to this agent.

5. C. W. Bruch: "Some Biological and Physical Factors in Dry Heat Sterilization (Resistance of Bacterial Sporeformers), A General Review," Life Science and Space Research, M. Florkin and A. Dollfus (ed), 1963

6. J. Opfell and C. Miller: "Cold Sterilization Techniques," Advances in App. Microbiology 7 (1966)

These are processes applied at temperatures below 50°C. They are applied to materials that are damaged or destroyed by heat sterilization processes. However, they may be chosen for heat-sterilizable materials to gain savings in money, time, or effort.

This study applies to chemical agents rather than processes based primarily on electromagnetic radiation, electron or particle beams, or even ultrasonic treatment.

7. Jaromir Synek: "Ethylene Oxide as a Sterilizing Agent," (1st Epidemiol Microbiol, Prague), Bull. Chim. Farm., 103 (11):790-803 (Ital), 1964

This paper is concerned with studies on the sterilizing properties of ethylene oxide. Previously sterilized materials, e.g., paper, cloth, glass, rubber, plastics, cotton, wool, etc, were impregnated with suspensions of Staphylococcus aureus, Escherichia coli, Mycobacterium phlei, and Bacillus mesentericus. Studies were conducted on the conditions necessary to completely sterilize these samples using a mixture of 10% ethylene oxide and 90% CO_2 at various pressures.

Complete sterilization required 24-hr exposure at 2.5 atm and 20 to 40°C or 18 hr (spores) and 6 hr (veg) at 40 to 50°C. It was concluded that time was important, and that it should be 6 to 12 hr for veg cells and 20 to 24 hr for spore formers, at 2.5 atm and 35 to 45°C. Also stated that the relative humidity should be low.

8. W. Haxeu and H. Hueck: "The Use of B-Propriolactone for the Sterilization of Heat Labile Materials," Antonie Leeuwenhoek J. Microbiol. Serol., 31(3):295-300 1965

A literature survey is given. Autoinactivation of this compound occurs.

9. I. Phillips and T. Tiylar: "Sterilization of Surgical Instruments in a 'Flash' Autoclave," Lancet 2, (7417):840-842 1965

10. P. M. Borich: "Antimicrobial Agents as Liquid Chemosterilizers," Biotechnol. Bioeng., 7(3):435-443 1965

Only a few antimicrobial agents are sporicidal and can be used as chemosterilizers. Various agents were tested for sporicidal activity.

11. Mark H. Chatigny: "Protection Against Infection in the Microbiological Laboratory: Devices and Procedures," Advances in App. Microbiology, 3:1 31-187 1961

This paper contains a very good section on decontamination, which includes the use of thermal, liquid, gas, or vapor phase, and UV irradiation. Also contains a section describing the advantages and disadvantages of many decontaminating agents.

12. John B. Opfell and Curtis E. Miller: "Cold Sterilization Techniques," Advances in App. Microbiology, 7:81-100 1965

Contains approximately 100 references. Areas discussed in this report include:

1) Sterilization of surfaces of solids including monitoring of sterilization processes;

2) Liquid and gaseous sterilants;

3) Internal sterilization - Demonstrating the effectiveness of processes to sterilize the interiors of solids, liquids and gases;

4) Self-sterilizing formulations for solids;

5) Self-sterilizing formulations for liquids and gases.

13. P. I. Howard: "Sterilization with Ethylene Oxide," (Inst. Cltn. Sci., Belfast, Ireland), J. Sci. Technol., 10(4):172-3 1964

This paper is mainly concerned with studies on precautions that should be used when working with ethylene oxide. Also, contains studies on the spectrum of organisms affected.

C. MICROBIOLOGY AND SPACE

1. Laboratory for Monitoring Contamination of Space Components (NASA), Quarterly Report, Phoenix Field Station, Technology Branch, Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, March 1966

Rigid plexiglass isolators were employed to simulate aerial fallout of micro-organisms onto surfaces. An 11x16x22-in. chamber was used. Aerosolized ethanol suspensions of B. subtilis indicated that a mean level of contamination can be controlled reliably.

Additionally, reports of experiments enumerated below are given:

1) Recovery of micro-organisms from solids;

2) Methods of removing contaminants from surfaces;

3) Microbial survival on surfaces;

4) Comparison of microbial contamination levels in hospital operating rooms and industrial clean rooms;

5) Recovery of sublethally heat damaged aerobic and anaerobic spores;

6) Comparison of standard plate count and membrane filter recovery methodology.

2. J. Hotchin et al.: "Survival of Micro-Organisms in Space," Nature, 206(4983):442-445 1965

The main environmental factors that were evaluated as possible causes of the loss of visibility of the exposed flight samples of micro-organisms are high vacuum, cosmic rays, ultraviolet-radiation, X-radiation and temperature extremes.

3. G. B. Phillips: "Absolute Barrier Concept in the Control of Micro-Organisms," Bact Proc, (1966)

Absolute barriers should allow no microbial interchange between protected and nonprotected environments; they aim at total containment. Postive pressure "product protection" barriers are needed for assembly of sterile spacecraft components. Cabinets and isolators may be used that isolate workers from the sterile area. Sterile rooms entered by workers wearing sterile ventilated suits also may be considered. Sterilization and decontamination agents are used for (1) initial sterilization of the barrier, (2) maintenance of sterility during use, and (3) treatment of supplies and equipment moved in and out of the barrier. Although sterilization by heat is best, chemicals such as ethylene oxide, peracetic acid, formaldehyde and beta-propriolactone find appropriate uses.

4. M. G. Koesterer: "Thermal Death Studies on Microbial Spores and some Considerations for the Sterilization of Spacecraft Components," Develop. Ind. Microbiol, 6:268-276

The thermal cycles required to reliably sterilize were defined. Various biological, chemical, and physical factors influencing the efficacy of dry heat as a sterilizing agent were investigated. Members of the aerobic mesophilic spore-forming group of bacteria, investigated in both pure culture and as they occur in soil, have been found to be highly resistant to destruction by dry heat.

5. T. R. Wilkinson: "Survival of Bacteria on Metal Surfaces," App. Microbiology, 14(3) 1966

Survival curves were determined for Serratia marcescens Sarcena lutea, Pasteurella tularensis, and P. pestis deposited from the airborne state onto metallic surfaces and subsequently stored at various humidities and temperatures. Cells of all species tested remained alive longest in a dry atmosphere, except that cells of Serratia marcescens survived best in a saturated atmosphere. Survival decreased most rapidly at the intermediate humidity level for three of the test organisms, yet P. tularensis died most rapidly in a saturated atmosphere. An increase in temperature decreased survival of P. pestis and P. tularensis. McDade and Hall reported 10^3 cells (Grom neg. sh.) stored on surfaces survived 48 hr at 25°C in the intermediate or high humidity range.

6. W. Pack, M. Christensen, and J. J. McDade: "Survival of Surface-Exposed Micro-Organisms in Spacecraft Assembly Area," Bact Proc (1966)

Results indicate that bacterial spores survive after several weeks of exposure to all of the above conditions (25 to 55°C and 10, 50, or 75% RH). Air velocities ranged from static through vertical (80 ft/min) or horizontal (110 ft/min) laminar airflow. Gram-positive cocci tend to survive desiccation under static conditions for periods in excess of one week, but die-away occurred with increasing rapidity above 50% RH or on exposure to the stream of laminar flow air. Gram-negative spp. were most susceptible to all of the above conditions, and die-away occurred within hours to days.

D. APPLICATION OF GNOTOBIOTIC TECHNOLOGY TO HUMANS

1. Sixth Quarterly Summary Report of Progress, Reduction of Microbial Dissemination, Germicidal Activity of Ethylene Oxide, Reduction of Microbial Contamination on Surfaces, Biophysics Section Technology Branch, Communicable Diseases Center, Public Health Service, U. S. Department of Health, Education, and Welfare, February 1966

Room-sized flexible film isolators were employed to determine the number and types of organisms shed by persons wearing various types of protective clothing. The use of the isolator precluded contamination with nonhuman organisms from the environment.

Results of this study demonstrated the superiority of this method of obtaining human-associated organisms. The advantages of the method included:

1) Short exposure periods;

2) Reduction of natural selection;

3) Reduced numbers of nonhuman-associated organisms;

4) Reduced variation in contamination loads;

5) Quantitation of total viable cell counts;

6) Accurate assay for spores and anaerobes.

Description of the isolator employed is not included. Sampling and assay methods are described in detail.

2. H. S. Gall and P. E. Riely: "Microbial Interaction Between Men and Their Environment in Simulated Space Chambers," Bact Proc (1966)

Interactions take place between microbial flora of men and their environment under simulated space conditions including confinements, limited personal hygiene procedures, wearing of space suits, diet, and altered atmospheric conditions.

3. J. A. Ulrich: "Aerobic Bacteria of the Human Skin," Bact Proc (1966)

Bacteria exist on and below the surface of human skin. It appears likely that many organisms reproduce in deeper sites and are then forced to the surface. A common pattern of distribution is noted in all normal individuals, but the level of the bacterial population is a characteristic of the individual. Levels remain constant over long periods, but can be temporarily changed by a variety of methods. Skin surface can be made relatively free from bacteria for short periods, but sterilization of the skin is not possible with present methods.

4. F. W. Thomac and M. Bengson: "Microbiological Constraints on Long-Term Manned Space Missions," Bact Proc (1966)

A strong possibility exists that bacteria will have to be added to the food or that controlled contamination of drinking water will be necessary to maintain a desirable intestinal population of micro-organisms.

5. J. J. Landy: "Treatment of the Burned Patient: Use of the Germfree Plastic Isolator as a Barrier Against Hospital Pathogens," Southern Med. J., 56(10):1084-1088 1963

Infection is a major cause of morbidity and mortality in burned patients. Strains of pathogenic bacteria prevalent in hospitals are the most important source of postburn infection. A system using germfree techniques in the treatment of burned patients is described.

6. T. D. Luckey: "Potential Microbial Hazards from Prolonged Isolation," Bact Proc (1966)

A dramatic decrease in the number of species in the gastrointestinal tract; many organisms considered to be indigenous to man will disappear. Expected morphological changes include decreased weight of lymph nodes, liver, liver and intestinal wall, and number of plasma cells. Chemical changes expected are: soft stools, odor changes reflecting one of the dominant microorganisms, decreased liver enzymes for detoxication, decreased complexity of metabolic products from intestinal putrefaction, and decreased synthesis of B-vitamins and vitamin K. Defense mechanisms will deteriorate at different rates. Susceptibility to virus and certain "nonpathogenic" bacteria would be increased. The accumulative effects could be lethal when astronauts return to this microbe dominated earth.

III. RÉSUMÉS OF ABSTRACTORS

This chapter contains the résumés and addresses of the personnel who prepared the abstracts in Chapter II. Those recipients who are interested in specific abstracts are welcome to contact the abstractors to discuss technical details.

DAROLD SMITH. Quality Engineer (794-5211, Ext 6268; Mail No. P-6700-3)

Education:

BS, University of Oklahoma, 1954
MS, University of Oklahoma, 1958
PhD, University of Texas, 1966

Dr. Smith represents the Quality Engineer with excellent capability to integrate this role into programs involving microbial contamination.

Dr. Smith's background includes research into bacterial contamination of fluids, experience in microbiology with the U. S. Public Health Service, as well as holding the position of Associate Professor of Biology at the University of Oklahoma.

ROBERT D. HOWELL. Senior Engineer (794-5211, Ext 6287; Mail No. P-6700-3)

Education:

BS in Biology and Chemistry, Northwestern State College, 1961
MS in Microbiology, University of Oklahoma, 1964
PhD in Microbiology, University of Oklahoma, 1966

Dr. Howell's background includes research in radiation biology, microbial physiology, and biochemistry. While in school, Dr. Howell worked part time with the Avco Corporation on studies dealing with biological radiation dosimeters.

Dr. Howell recently joined the Martin Company, and has participated in planning and analysis of biomedical experiments for the Apollo Applications Program. He is currently working in Technical Operation Services on studies dealing with spacecraft hardware sterilization.

Publications:

"Environmental Factors Affecting Photoprotection Against X-Ray Damage in Staphylococcus Aureus." Bacteriological Proceedings, 1964.

The Effect of X-Radiation on Respiration of Staphylococcus Aureus. Thesis for Master of Science Degree, University of Oklahoma, 1964.

Changes in Metabolism Associated with Changes in Radioresistance in Staphylococcus Aureus. Dissertation for Doctor of Philosophy Degree, University of Oklahoma, 1966.

Member:

Sigma Xi; Phi Sigma; Oklahoma Academy of Science; and American Society for Microbiology.

HARRY M. KAUFMAN. Microbiology Supervisor (794-5211, Ext 6288; Mail No. P-6700-3)

Education:

BS in Biological Science, Texas Western College, 1961
MS in Microbiology, Arizona State University, 1964

Mr. Kaufman has achieved a high level of qualification in the field of microbiology in a comparatively short time. He is outstanding in his understanding of this field and its requirements in relation to the assembly of spacecraft and their components.

Mr. Kaufman has conducted research in CBN warfare and detection. He has participated in planning the biomedical experiment analysis for the Apollo Applications Program and has been responsible for the sterilization task evaluation and experiment design on the Voyager proposal.

Publications:

"The Effect of Hypothermia on the in vivo Phagocytosis of D. pneumoniae by Immune and Non-Immune Rabbits." American Society of Microbiology National Meeting in Atlantic City, N. J., April 1965.

Member:

American Society for Microbiology; Aerospace Medical Association; and Aerospace Industries Life Sciences Association.

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